In re Application of Roy Duncan

PATENT Attorney Docket No. 78973-1C

Application No.: to be assigned

Filed: Continuation of SN 08/965,708

filed November 7, 1997

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In the Claims:

Please cancel claims 1 to 3 and 29 to 40.

Claims 5 to 28 have been amended and claims 43 to 77 have been added. Claims 4 to 28 and 41 to 77 are now pending.

Amended claims 5 to 28 and new claims 43 to 79 are as follows:

5. [Amended] The protein of claim 4 having an amino acid sequence substantially the same as set forth in SEQ ID NO: 14 [BRV].

6. [Amended] The protein of claim 4 having the amino acid sequence set forth in SEQ ID NO: 14 [BRV].

7. [Amended] An antibody raised against the protein of claim 43.

8. [Amended] An antibody raised against the protein of claim 4.

9. [Amended] An isolated nucleic acid encoding the protein of claim 43.

10. [Amended] An isolated nucleic acid according to claim 9 having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-1607 of SEQ ID NO: 1 [ARV1],

nucleotides 25-1607 of SEQ ID NO: 5 [ARV2],

nucleotides 27-1579 of SEQ ID NO: 9 [NBV], or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

11. [Amended] An isolated and purified nucleic acid, or functional fragment thereof encoding the protein of claim 43, wherein the nucleic acis is selected from the group consisting of:

(a) DNA encoding the amino acid sequence set forth in SEQ ID NO: 2, SEQ ID NO: 6 or SEQ ID NO: 10, or

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- (b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes a biologically active fusion protein, or
- (c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes a biologically active fusion protein.
- 12. [Amended] An isolated nucleic acid according to claim 9 operatively associated with an inducible promoter.
- 13. [Amended] An isolated nucleic acid encoding the protein of claim 4.
- 14. [Amended] The isolated nucleic acid of claim 13 having a contiguous nucleotide sequence substantially the same as:

nucleotides 25-832 of SEQ ID NO: 13 [BRV], or

variations thereof which encode the same amino acid sequence, but employ different codons for some of the amino acids, or splice variant nucleotide sequences thereof.

- 15. [Amended] An isolated and purified nucleic acid, or functional fragment thereof encoding the protein of claim 4, wherein the nucleic acid is selected from the group consisting of:
- (a) DNA encoding the amino acid sequence set forth in SEQ ID NO: 14, or
- (b) DNA that hybridizes to the DNA of (a) under moderately stringent conditions, wherein said DNA encodes a biologically active fusion protein, or
- (c) DNA degenerate with respect to either (a) or (b) above, wherein said DNA encodes a biologically active fusion protein.
- 16. [Amended] The isolated nucleic acid of claim 13 operatively associated with an inducible promoter.
- 17. [Amended] A cell containing the protein of claim 43.
- 18. [Amended] The cell containing the protein of claim 4.

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- [Amended] The cell containing the nucleic acid of claim 9. 19.
- [Amended] The cell containing the nucleic acid of claim 12. 20.
- [Amended] The cell containing the nucleic acid of claim 13. 21.
- [Amended] The cell containing the nucleic acid of claim 16. 22.
- [Amended] Liposomes containing the protein of claim 43, 23.
- [Amended] Liposomes containing the protein of claim 4. 24.
- [Amended] Liposomes containing the nucleic acid of claim 9. 25.
- [Amended] Liposomes containing the nucleic acid of claim 13. 26.
- [Amended] A method for producing the protein of claim 43, said method 27. comprising the step of expressing a nucleic acid encoding said protein in a suitable host.
- [Amended] A method for producing the protein of claim 4, said method 28. comprising the step of expressing a nucleic acid encoding said protein in a suitable host.
- [New] An isolated protein which: 43.
- (a) is a membrane fusion protein;
- (b) comprises a transmembrane domain; and
- (c) has at least 33% amino acid sequence identity to a protein which:
 - is encoded by a polynucleotide from the genome of Reoviridae;
 - is a membrane fusion protein, and (ii)
 - has a molecular weight of about 11 kDa. (iii)
- [New] The isolated protein of claim 43 which:
- (a) is encoded by a polynucleotide from the genome of Reoviridae; and
- (b) has molecular weight of about 11 kDa.
- [New] The protein of claim 43 which has less than 100 amino acids or 45. has about 100 amino acids.
- [New] The protein of claim 43 which contains a cluster of positive amino 46. acid residues, wherein the cluster is located on the C-terminal side of the

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transmembrane domain and comprises at least 4 positive residues within the 20 residues flanking the transmembrane domain at the C-terminal side.

- 47. [New] The protein of claim 43 which lacks a signal peptide.
- 48. [New] The protein of claim 43 which contains 4 cysteine residues at conserved positions relative to SEQ ID NO:2 (ARV1); SEQ ID NO:6 (ARV2); and SEQ ID NO:10 (NBV).
- 49. [New] The isolated protein of claim 43 which has at least 33% amino acid sequence identity to a polypeptide selected from the group consisting of:
- (a) a polypeptide of SEQ ID NO:2 (ARV1);
- (b) a polypeptide of SEQ ID NO:6 (ARV2); and
- (c) a polypeptide of SEQ ID NO:10 (NBV).
- 50. [New] The protein of claim 49 which has at least 33% amino acid sequence identity to the polypeptide of SEQ ID NO:2 (ARV1).
- 51. [New] The protein of claim 49 which has at least 33% amino acid sequence identity to the polypeptide of SEQ ID NO:6 (ARV2).
- 52. [New] The protein of claim 49 which has at least 33% amino acid sequence identity to the polypeptide of SEQ ID NO:10 (NBV).
- 53. [New] The protein of claim 49 which comprises a cluster of positive amino acid residues, wherein the cluster is located on the C-terminal side of the transmembrane domain and comprises at least 4 positive residues within the 20 residues flanking the transmembrane domain at the C-terminal side.
- 54. [New] The protein of claim 49 which lacks a signal peptide.
- 55. [New] The protein of claim 49 which comprises 4 cysteine residues at conserved positions relative to SEQ ID NO:2 (ARV1); SEQ ID NO:6 (ARV2); and SEQ ID NO:10 (NBV).
- 56. [New] An isolated protein comprising the sequence selected from the group consisting of: SEQ ID NO:2(ARV1), SEQ ID NO:6(ARV2) and SEQ ID NO:10(NBV).
- 57. [New] An isolated protein which:

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- is a membrane fusion protein; (a)
- comprises a transmembrane domain; (b)
- has a molecular weight of about 15 kDa; and (c)
- is encoded by a polynucleotide from the genome of Reoviridae. (d)
- [New] A method to promote membrane fusion, said method comprising the step of contacting the membranes to be fused with the protein of claim 43 for a time and under conditions effective to promote membrane fusion.
- [New] A method to promote membrane fusion, said method comprising the step of contacting the membranes to be fused with the protein of claim 57 for a time and under conditions effective to promote membrane fusion.
- [New] A method to promote membrane fusion, said method comprising the step of contacting the membranes to be fused with a membrane fusion protein for a time and under conditions effective to promote membrane fusion, wherein the membrane fusion protein is encoded by a polynucleotide of the genome of a fusogenic member of the family Reoviridae or is substantially the same as the membrane fusion protein encoded by a polynucleotide of the genome of a fusogenic member of the family Reoviridae.
- [New] The method of claim 60 wherein the fusogenic member of the 61. family Reoviridae is selected from: ARV, NBV and BRV.
- [New] The method of claim 60 wherein the membranes are cell 62. membranes, liposome membranes or proteoliposome membranes.
- [New] The method of claim 60 wherein the membranes are the cell membrane of an immortalized myeloma cell and the cell membrane of a primary B cell or T cell.
- [New] The method of claim 63 wherein the immortalized myeloma cell is human or mouse, and wherein the primary B cell or T cell is a purified spleen cell from an immunized mammal.
- [New] The method of claim 60 wherein the membranes to be fused are the cell membrane of an immortalized myeloma cell and the cell membrane of a primary B cell or T cell, and wherein the membrane fusion protein has an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10, 14, and substantially the same sequences thereof.

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[New] A method to promote membrane fusion between a first membrane 66. and a second membrane, said method comprising:

introducing a fusogenic protein, in an amount sufficient to effect membrane fusion, into the first membrane; and then,

contacting the second membrane with the first membrane for a time and under conditions effective to promote membrane fusion between the first membrane and the second membrane;

wherein the first membrane is selected from the group consisting of:

a liposome membrane; (i)

a proteoliposome membrane; and (ii)

a membrane of a cell; (iii)

and wherein the fusogenic protein either:

is encoded by a polynucleotide of the genome of Reoviridae; and

has a molecular weight of about 11 kDa; and (ii)

is less than 100 amino acids or is about 100 amino acids; (iii) or wherein the fusogenic protein:

is encoded by a polynucleotide of the genome of Reoviridae; and (i)

has a molecular weight of about 15 kDa; and (ii)

is less than 150 amino acids or is about 150 amino acids. (iii)

- [New] The method according to claim 66 wherein the first membrane is 67. the liposome membrane or the proteoliposome membrane.
- [New] The method according to claim 67, wherein the step of introducing 68. the fusogenic protein comprises incorporating the fusogenic protein into the liposome membrane or the proteoliposome membrane.
- [New] The method according to claim 66 wherein the first membrane is 69. the cell membrane.
- [New] The method according to claim 69, wherein the step of introducing 70. the fusogenic protein comprises the step of introducing into the cell an expression vector comprising a polynucleotide which encodes the fusogenic protein, wherein the vector is free of full-length reovirus genome.
- [New] A method to promote membrane fusion between a first membrane 71. and a second membrane, said method comprising contacting the first or

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second membrane or both membranes with an effective amount of a proteinliposome complex for a time and under conditions effective to promote membrane fusion between the first membrane and the second membrane, wherein the protein-liposome complex contains a fusogenic protein; and

wherein the fusogenic protein either:

- is encoded by a polynucleotide of the genome of Reoviridae; (i)
- has a molecular weight of about 11 kDa; and (ii)
- is less than 100 amino acids or is about 100 amino acids; (iii) or wherein the fusogenic protein:
- is encoded by a polynucleotide of the genome of Reoviridae; (i)
- has a molecular weight of about 15 kDa; and (ii)
- is less than 150 amino acids or is about 150 amino acids. (iii)
- [New] The method according to claim 66 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.
- [New] The method according to claim 67 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.
- [New] The method according to claim 68 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.
- [New] The method according to claim 69 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 75. 10 and 14.
- [New] The method according to claim 70 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.
- [New] The method according to claim 71 wherein the fusogenic protein comprises an amino acid sequence selected from any one of: SEQ ID NOs: 2, 6, 10 and 14.